REMARKS

These remarks are in response to the Office Action dated June 29, 2006. Applicants have canceled claims 1-15, 17 and 19-53 without prejudice to Applicants' right to prosecute the canceled subject matter in any divisional, continuation, continuation-in-part or other application. Applicants have amended claim 16. New claims 54, 55 and 56 have been added. Support for the amendments and new claims can be found throughout the specification and claims as originally filed.

No new matter is believed to have been introduced. Claims 16, 54, 55 and 56 are pending and at issue. Applicants request reconsideration of the pending claim.

Rejections Under 35 U.S.C. § 112, first paragraph

Enablement

Claims 16 and 17 stand rejected under 35 U.S.C. § 112, first paragraph, as the claims allegedly fail to comply with the enablement requirement. This rejection is moot with regard to canceled claim 17. Applicants traverse this rejection as applied to amended claim 16.

Briefly, the Office alleges that undue experimentation would be needed to determine which agents effect CG activity in the claimed method. Applicants note that claim 16 has been amended to recite agents that include peptides comprising exoloop 1, exoloop 2 and/or exoloop 3 domain of the LHR (i.e., LHR^{exo1}, LHR^{exo2}, and/or LHR^{exo3}). These domains were previously identified as components of the C-terminal "endodomain" of the leutinizing hormone receptor (LHR). However, prior to the data provided in the present specification, it was not known that these domains interacted with chorionic gonadotropin (CG) when CG bound to intact LHR. Further, it was not known that peptide fragments comprising the LHR^{exo1}, LHR^{exo2}, or LHR^{exo3} domains could bind to CG. In support, Applicants point to paragraph [0304] of the published application (US Patent Application Publication No. 2005/0142135) which recites, in part:

> The specific photoaffinity labeling of hCG by ABG-^{125} I-LHR^{exo3} shows the direct interaction, and this result is consistent with the inhibition of hCG binding to LHR by the peptide, albeit with low affinity. This is a novel and important observation and provides a new insight into the mechanism of the signal generation, particularly considering the recent reports that the exodomain modulates the signal generation by

> interacting with exoloop 2. Therefore, exoloop 3 interacts with the exodomain, in addition to the interaction with hCG, and participate in the signal generation. Nonlabeled exoloop 2 peptide blocked the labeling of both hCGα and hCGβ as did nonlabeled exoloop 3 peptide, suggesting the competitive nature of their interactions with hCG. Not only exoloop 2 but also the LRR 4 peptide, LHR^{96-115} and the hinge region peptide, $LHR^{246-269}$, inhibited the labeling. In contrast, the exoloop 1 peptide and the N-terminal peptide, LHR^{17-36}, were less potent in the inhibition.

Applicants note that the claimed method utilizes peptides that include amino acid residues constituting the LHR^{exo1}, LHR^{exo2}, and LHR^{exo3} domains. The sequences of these domains were previously disclosed (see e.g., the figure legend associated with Figure 11 as set forth in Exhibit A attached hereto). The peptides that include these domains are limited to about 24-32, 21-29 or 12-20 residues in length and having at least 95% sequence identity to the amino acid sequence of the LHR^{exo1}, LHR^{exo2}, or LHR^{exo3} domain. Support for the recitation of "95%" sequence identity can be found at paragraph [0107] of the published application (see US Patent Application Publication No. 2005/0142135).

Applicants note that the specification does not teach the specific amino acids that can be added to the LHR^{exo1}, LHR^{exo2}, or LHR^{exo3} to arrive at peptides having a length about 24-32, 21-29 or 12-20 residues, respectively. However, given the information provided in the specification for identifying peptides that bind to GC (see e.g., paragraphs [0283] - [0304] of US Patent Application Publication No. 2005/0142135) the amount of experimentation required to make such peptides would not be undue. For the purposes of satisfying 35 U.S.C. §112, the disclosure of a natural sequence in and of itself is generally sufficient for disclosing a variance for that sequence (see Ex parte Bandman, No. 2004-2319, (BPAI January 2005) (Non-Precedential). Applicants submit that the novelty of the claimed method lies not in the use of novel peptide sequences, but instead relies on the novel observation that exoloop domains of the LHR interact with GC. Accordingly, the moderate sequence variations encompassed by the peptides utilized in the claimed method are well within the ability of the skilled artisan to manufacture because they are inherently limited to those that bind to CG and inhibits CG interaction with the exoloop 1, exoloop 2 or exoloop 3 domain of the LHR.

Written Description

Claims 16 and 17 stand rejected under 35 U.S.C. § 112, first paragraph, as the claims allegedly fail to comply with the written description requirement. This rejection is moot with regard to canceled claim 17. Applicants traverse this rejection as applied to amended claim 16.

To advance prosecution Applicants have amended claim 16 to be limited to those agents that include a subset of peptides encompassing the LHR^{exo1}, LHR^{exo2}, and LHR^{exo3} domains, and limited variants thereof. As previously noted, the novelty of the claimed method lies not in the use of novel peptide sequences, but instead relies on the novel observation that exoloop domains of the LHR interact with GC. Accordingly, a description of the complete structure of every sequence variation encompassed by the peptides utilized in amended 16 is not required in order to satisfy the written description requirement of §112, first paragraph. All that is required is that the applicant convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, and that the invention, in that context, is whatever is now claimed. Applicants submit that these requirements are met in view of the amendments to claim 16 and in light of the information provided in the specification.

Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. §112, first paragraph be withdrawn.

Claim Rejections Under 35 U.S.C. § 102(b)

Claims 16-17 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Talwar et al. (*Proc. Natl. Acad. Sci.* 91:8532-8536 (1994)) ("Talwar"). This rejection is moot with regard to canceled claim 17. Applicants traverse this rejection as applied to amended claim 16.

Talwar purportedly teaches a composition comprising a biologically active fragment of CG (βhCG) that neutralizes the bioactivity of hCG and reduces incidence of conception. Applicants submit that Talwar fails to teach an agent comprising or consisting of a peptide encompassing variations of the LHR^{exo1}, LHR^{exo2}, or LHR^{exo3} domains as currently recited in amended claim 1. The cited reference could not anticipate the claimed method because Applicants were the first to identify any

interaction between LHR exoloops 1, 2, and 3 with a GC hormone. Accordingly, Applicants request that this rejection be withdrawn.

CONCLUSION

It is respectfully submitted that all rejections have been overcome by the above amendments. Thus, Notice of Allowance is respectfully requested.

In the event that there are any questions relating to this Amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (858) 509-7318 so that prosecution of the application may be expedited.

Respectfully submitted,

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Exhibit A

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Hormone Interactions to Leu-rich Repeats in the Gonadotropin Receptors

III. PHOTOAFFINITY LABELING OF HUMAN CHORIONIC GONADOTROPIN WITH RECEPTOR LEU-RICH REPEAT 4 PEPTIDE*

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Human chorionic gonadotropin (hCG) binds to the extracellular N-terminal domain, exodomain, of its receptor, and the resulting hCG-exodomain complex is thought to modulate the membrane associated domain, endodomain, of the receptor to generate hormone signal. The bulk of the exodomain is speculated to assume a crescent structure consisting of eight to nine Leu-rich repeats (LRRs), which may provide the hormone contact sites. Unfortunately, little experimental evidence is available for the precise hormone contact points in the exodomain and the endodomain. The two preceding articles (Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) *J. Biol. Chem.* 276, 3426–3435; Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) *J. Biol. Chem.* 276, 3436–3442) show that putative LRR2 and LRR4 are crucial for hormone binding. In particular, the N-terminal region of LRR4 assumes the hydrophobic core of the LRR4 loop, whereas the C-terminal region is crucial for signal generation. However, it is unclear whether LRR4 interacts hCG and the endodomain and how it might be involved in signal generation. In this article, our affinity labeling results present the first evidence that the N-terminal region of LRR4 interacts with hCG, preferentially the hCGα subunit and that the hCG/LRR4 complex interacts with exoloop 2 of the endodomain. This interaction offers a mechanism to generate hormone signal.

The luteinizing hormone/chorionic gonadotropin receptor (LHR)[1] consists of an extracellular N-terminal half (exodomain) and a membrane-associated C-terminal half (endodomain) (1, 2). The ~350-amino acid-long exodomain has high affinity hormone contact sites (3–5) and shows eight to nine repeats of 22–29 amino acids with several conserved Leu/Ile residues (1, 6–10). These Leu/Ile-rich repeats (LRRs) represent a common structural motif found in a large family of proteins, which includes glycoprotein hormone receptors (11). In the crystal structure of ribonuclease inhibitors, the LRRs assume the horseshoe structure in which individual LRRs form a loop consisting of a β strand connected to parallel α helices. The β strands in ribonuclease inhibitors are involved in the interaction with ribonuclease. However, it is unclear whether the putative LRR sequences of LHR and other glycoprotein hormone receptors are indeed LRRs and function as such. In the preceding articles (12, 13), we have shown that some, but not all, LRRs of LHR and the follicle-stimulating hormone receptor are crucial for hormone binding. In particular, LRR2 and LRR4 of LHR are most crucial, but it is unclear whether these LRRs make direct contacts with the hormone. In this article, the evidence is presented for the interaction of the residues around the Leu-Ser-Ile motif, the putative β strand, in LRR4 with hCG, in particular with the hCGα subunit. In addition, our data suggest the interaction of the LRR4-hCG complex with the endodomain, in particular exoloop 2, which is likely to modulate signal generation.

EXPERIMENTAL PROCEDURES

Materials—The *N*-hydroxysuccinimide (NHS) ester of 4-azidobenzoic acid (AB) was synthesized as described previously (14). The *N*-hydroxysulfosuccinimide esters of ethylene glycolbis(sulfosuccinimidylsuccinate) (SES) were purchased from Pierce. The hCG CR 127 and hCG subunits were supplied by the National Hormone and Pituitary Program. Denatured hCG was prepared by boiling hCG in 8 M urea for 30 min. Receptor peptides were synthesized and *N*-acetylated and *C*-amidated by Biosynthesis (Lewisville, TX). They were purified on a Vydac C_{18} high performance liquid chromatography column using a solvent gradient from 100% of 0.1% trifluoroacetic acid in water to 20% of 0.1% trifluoroacetic acid in water and 80% 1-propanol. The peptide mimics include the wild type receptor peptide corresponding to the LHR sequence of Asn^{96}–Asp^{115} (LHR^{96-115}), a mutant LHR^{96-115} with Leu^{103}Ala and Ile^{105}Ala mutations ($LHR^{96-115(L103A/I105A)}$), a mutant LHR^{96-115} with the $Lys^{101} \rightarrow$ Ala mutation ($LHR^{96-115(K101A)}$), a mutant LHR^{96-115} with the $Lys^{112} \rightarrow$ Ala mutation ($LHR^{96-115(K112A)}$), a wild type peptide encompassing the sequence upstream of LHR^{96-115} (LHR^{85-104}), and a wild type peptide covering the sequence downstream of LHR^{96-115} ($LHR^{113-132}$).

Derivatization and Radioiodination of Peptides—NHS-AB was freshly dissolved in dimethyl sulfoxide to a concentration of 50 mM in 0.1 M sodium phosphate (pH 7.5) to a concentration of 20 mM. This reagent solution was immediately used to derivatize receptor peptides. In the dark, 10 μl of NHS-AB was added to 30 μg of LHR^{96-115} in 40 μl of 0.1 M sodium phosphate (pH 7.5). The mixture was incubated for 30 min for NHS-AB or 60 min for NHS-AB at 25 °C. The following were added to the derivatization mixture: 1 mCi of $Na^{125}I$ in 10 μl of 0.1 M NaOH and 7 μl of chloramine T (1 mg/ml) in 10 mM Na_2HPO_4 and 0.9% NaCl (pH 7.4) (PBS). After 20 s, 7 μl of sodium metabisulfite (2.5 mg/ml) in PBS was introduced to terminate radioiodination. Derivatized and radioiodinated AB-^{125}I-LHR^{96-115} solution was mixed with 60 μl of 16% sucrose solution in PBS and fractionated on Sephadex Superfine G-10 column (0.6 × 15 cm) using PBS.

Affinity Cross-linking of ^{125}I-LHR^{96-115} to hCG—Disposable glass tubes were siliconized under dimethyldichlorosilane vapor overnight and autoclaved. In each siliconized tube, 20 μl of PBS, hCG (70 ng in 10 μl PBS), and ^{125}I-LHR^{96-115} (100 ng in 10 μl of PBS) were mixed and

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[1] The abbreviations used are: LH, luteinizing hormone; LHR, LH receptor; CG, choriogonadotropin; h, human; LRR, Leu-rich repeat; AB, 4-azidobenzoyl; NHS, *N*-hydroxysuccinimide; SES, ethylene glycolbis(sulfosuccinimidylsuccinate); PBS, phosphate-buffered saline.

incubated in 37 °C for 90 min. After incubation, 3 μl of 0.1 mM of SES in dimethyl sulfoxide was added to each tube and further incubated at 25 °C for 20 min. The cross-linking reaction was terminated by adding 3 μl of 5 mM Gly in PBS. The samples were boiled for 2 min in 2% sodium dodecyl sulfate, 100 mM dithiothreitol, and 8 M urea. The solubilized samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried on filter paper, which was exposed to a molecular imaging screen (Bio-Rad) overnight. The imaging screen was scanned on a model GS-525 Molecular Imager System Scanner (Bio-Rad), and radioactive band intensity was analyzed using Image Analysis Systems, Version 2.1 (Bio-Rad). Gels were exposed to X-Omat x-ray film at −75 °C for ~4 days.

Photoaffinity Labeling of hCG—The following solutions were sequentially introduced to siliconized glass tubes: 20 μl of PBS, 10 μl of hCG (10 ng/μl) in PBS, and 10 μl of AB-^{125}I-LHR$^{96-115}$ (10 ng/μl) in PBS. The mixtures were incubated at 37 °C for 90 min in the dark, irradiated with a Mineralight R-52 UV lamp for 3 min as described previously (14), and solubilized in 2% SDS, 100 mM dithiothreitol, and 8 M urea. The samples were electrophoresed on 8–12% polyacrylamide gradient gels. Gels were dried on filter paper and processed as described above.

Competitive Inhibition of Affinity Labeling of hCG—Competitive inhibition experiments were carried out as described for the affinity cross-linking and photoaffinity labeling experiments, except that 10 μl instead of 20 μl of PBS was introduced to each tube, and the mixture was incubated with 10 μl of increasing concentrations of nonradioactive wild type or mutant LHR$^{96-115}$.

Inhibition of ^{125}I-hCG binding to LHR—A human embryonic kidney 293 cell line stably expressing human LHR was incubated with 100,000 cpm of ^{125}I-hCG in the presence of increasing concentrations of nonradioactive wild type or mutant LHR$^{96-115}$ peptides as described previously (15). After several times washing the cells, the radioactivity associated with the cells was counted, and percent bound ^{125}I-hCG was plotted against the nonradioactive receptor peptides. The results were converted to Scatchard plot by plotting bound/free peptide *versus* bound peptide. The plot was used to calculate the K_d value following the Scatchard equation (16).

RESULTS

In the preceding articles (12, 13), we showed the crucial roles of LRRs of LHR in hormone binding, particularly LRR4. This raises the question as to whether the LRRs directly interact with the hormone or indirectly influence the hormone/receptor interaction by impacting the global structure of the receptor exodomain. To examine these possibilities a peptide mimic corresponding to the receptor sequence encompassing the β-stranded Leu^{103}–Ile^{105}, LHR$^{96-115}$, was synthesized and tested for its ability to bind and affinity label hCG. For affinity labeling, we employed two complementary affinity labeling methods. In the first approach, ^{125}I-LHR$^{96-115}$ incubated with hCG, and the resulting ^{125}I-LHR$^{96-115}$-hCG complexes were cross-linked using SES, a homobifunctional reagent that is capable of cross-linking two amino groups up to 13 Å apart (17). In the second approach, ^{125}I-LHR$^{96-115}$ was derivatized with AB, an UV-activable reagent, to produce AB-^{125}I-LHR$^{96-115}$ and incubated with hCG. The resulting ^{125}I-LHR$^{96-115}$-hCG complex was irradiated with UV to photoaffinity label hCG with AB-^{125}I-LHR$^{96-115}$. The advantages and disadvantages of both methods will be discussed later.

To determine whether AB-^{125}I-LHR$^{96-115}$ and ^{125}I-LHR$^{96-115}$ would bind and label hCG, they were incubated with hCG and treated with UV or SES, respectively. The samples were solubilized in SDS under the reducing condition and electrophoresed, as described under "Experimental Procedures." The autoradiographic phosphoimage of the gel shows that both AB-^{125}I-LHR$^{96-115}$ and ^{125}I-LHR$^{96-115}$ labeled both the α and β subunits in hCG (Fig. 1). In addition, the hCG αβ dimer was cross-linked and labeled with ^{125}I-LHR$^{96-115}$ when the ^{125}I-LHR$^{96-115}$-hCG complex was treated with SES. The positions of hCGα, hCGβ, and the hCGαβ dimer were determined by comparing the respective positions of ^{125}I-hCGα, ^{125}I-hCGβ, and the cross-linked ^{125}I-hCG αβ dimer on the autoradiograph (Fig. 1, *lanes 1* and *5*).

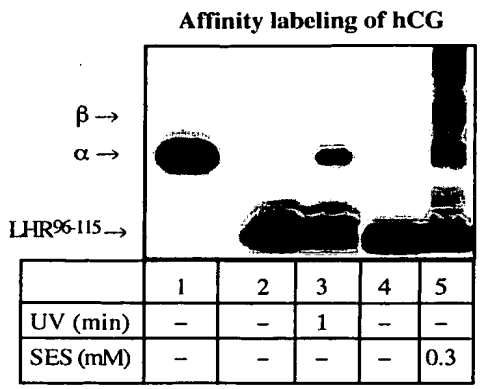

FIG. 1. **Autoradiograph of affinity-labeled hCG subunits.** hCG was incubated with AB-^{125}I-LHR$^{96-115}$ (*lanes 2* and *3*) or ^{125}I-LHR$^{96-115}$ (*lanes 4* and *5*) and treated with UV for 1 min (*lane 3*) or 0.3 mM SES (*lane 5*), respectively, as described under "Experimental Procedures." After electrophoresis of the samples, the gel was dried and autoradiographed using PhosphoImager. *Lane 1*, ^{125}I-hCG showing radiolabeled α and β subunits as standards.

Cross-linking of ^{125}I-LHR$^{96-115}$ to hCG—As a first step to determine the specificity of affinity labeling, ^{125}I-LHR$^{96-115}$ was incubated with hCG and treated with increasing concentrations of SES. Electrophoresis of the treated hCG/^{125}I-LHR$^{96-115}$ mixture (Fig. 2*A*) shows that ^{125}I-LHR$^{96-115}$ was cross-linked to hCGα, hCGβ, and the hCGαβ dimer. The extent of cross-linking was dependent on the SES concentration, reaching the maximum level at 0.3–1 mM SES. Under this condition, ~20% of ^{125}I-LHR$^{96-115}$ was cross-linked to hCGα and ~10% to hCGβ. At higher SES concentrations, for example 10 mM, the extent of cross-linking decreased. This decrease was due to noncross-linking, monofunctional reactions (only one of the two NHS groups reacting with a target amino group while the other NHS group undergoing hydrolysis) of excess SES with ^{125}I-LHR$^{96-115}$, hCG, and its subunits (18). In conclusion, our results indicate that ^{125}I-LHR$^{96-115}$ was covalently cross-linked to hCGα and hCGβ. Furthermore, either or both amino groups of Lys^{101} and Lys^{112} of ^{125}I-LHR$^{96-115}$, the only amino groups of the peptide, were cross-linked to an amino group(s) of either hCGα or hCGβ. The distance between the pair of two cross-linked amino groups is expected to be <13 Å.

Saturable Cross-linking of ^{125}I-LHR$^{96-115}$ to hCG—To determine whether the cross-links are specific between the receptor peptide and hCG, cross-linking was performed under increasing concentrations of ^{125}I-LHR$^{96-115}$ while maintaining hCG at a constant concentration (Fig. 2*B*). Conversely, ^{125}I-LHR$^{96-115}$ and hCG were cross-linked at increasing concentrations of hCG and a constant concentration of ^{125}I-LHR$^{96-115}$ (Fig. 2*C*). If cross-links are specific, they should reach saturation under both conditions. The results indeed show plateaus under both conditions, an indication of saturable and specific cross-linking. This specific cross-linking is not expected to occur with peptides that do not recognize hCG.

Photoaffinity Labeling of hCG—Despite the indication for saturable and specific cross-links between the receptor peptide and hCG, there were a series of minor cross-linked complexes larger than the complex of ^{125}I-LHR$^{96-115}$ and the hCG dimer. They suggest that a minor population of the ^{125}I-LHR$^{96-115}$-hCG dimer complex may be further cross-linked to another hCG subunit or hCG dimer. Although this is not entirely unexpected, as random collisional cross-links are possible (18), it raises a concern on the specificity of homobifunctional cross-links between ^{125}I-LHR$^{96-115}$ and hCG. A simple way to reduce or eliminate such random collisional cross-links is photoaffinity

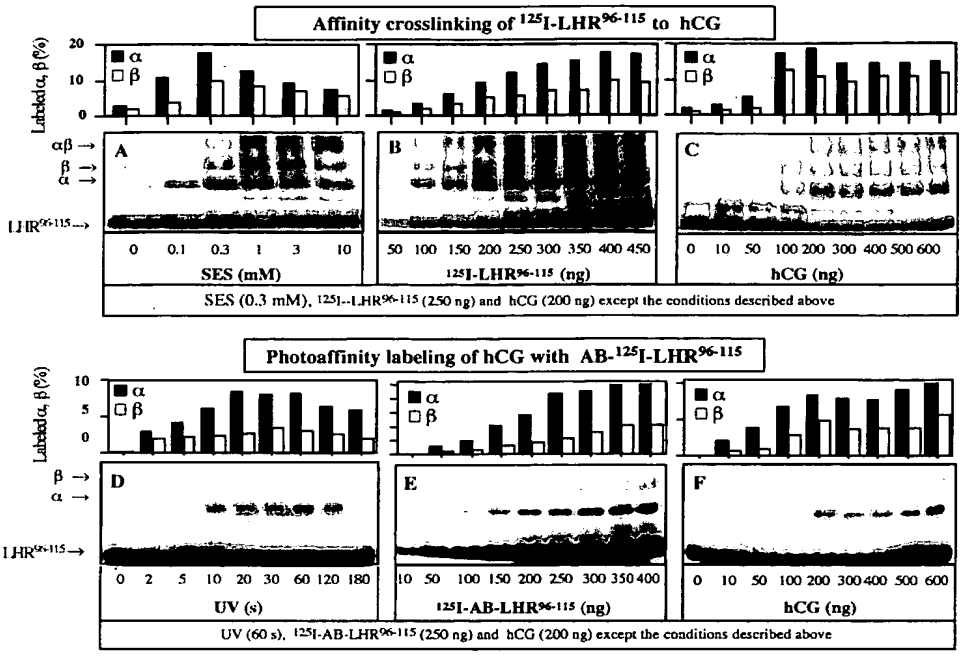

FIG. 2. **Affinity labeling is saturable.** hCG was incubated with ^{125}I-LHR$^{96-115}$ and treated with SES (*A–C*). In this series of experiments, the increasing concentrations of SES (*A*), ^{25}I-LHR$^{96-115}$ (*B*), or hCG (*C*) were applied, while the other two conditions were kept constant. In *D–F*, hCG was incubated with AB-^{125}I-LHR$^{96-115}$ and irradiated with UV. In this series, the UV irradiation time (*D*), AB-^{125}I-LHR$^{96-115}$ concentration (*E*), or hCG concentration (*F*) was varied, while the other two factors were kept constant. After electrophoresis of the samples, gels were dried on filter paper and exposed to a molecular imaging screen (Bio-Rad) overnight. The imaging screen was scanned on a model GS-525 Molecular Imager System Scanner (Bio-Rad), and the radioactive band intensity was analyzed using Image Analysis Systems Version 2.1 (Bio-Rad). Gels were also exposed to X-Omat x-ray film at −75 °C for ~4 days. The *bar graphs* show the percent radioactivity of the α band and the β band in a gel lane.

labeling (18). To photoaffinity label hCG with ^{125}I-LHR$^{96-115}$, the receptor peptide was derivatized with AB to produce AB-^{125}I-LHR$^{96-115}$. When the derivatized peptide binds to hCG and is irradiated with UV, the cross-link will be restricted between ^{125}I-LHR$^{96-115}$ and hCGα or between ^{125}I-LHR$^{96-115}$ and hCGβ. The reagent, however, will not be able to cross-link an hCG subunit to another. AB can reach and label target molecules up to 7 Å (19). The distance is considerably shorter than the maximum cross-linkable 13 Å of SES and therefore, the labeling reaction by AB is more restricted than the cross-linking reaction by SES. On the other hand, cross-linking with SES can be useful when AB attached at the contact point might interfere with the interaction.

As shown in Fig. 2, *D–F*, AB-^{125}I-LHR$^{96-115}$ was capable of photoaffinity labeling either hCGα or hCGβ but not both subunits at the same time. The labeling is generally confined to hCGα with the labeling of hCGβ being faint. This result is consistent with the SES cross-linking results. One possible explanation is that the peptide is bound closer to α than β. The labeling required UV irradiation and was dependent on the irradiation time, reaching the maximum labeling after 30~60-s irradiation. This UV dependence clearly indicates photoaffinity labeling. In addition, the preferential labeling of hCGα without simultaneously labeling of both subunits suggests a labeling specificity. To further examine the specificity of photoaffinity labeling, the concentration of either hCG or the peptide derivatives was changed. When a constant concentration of AB-^{125}I-LHR$^{96-115}$ was incubated with increasing concentrations of hCG, the intensity of labeled hCGα and β bands gradually increased and plateaued (Fig. 2*E*). A similar result was obtained in a converse experiment when a constant amount of hCG was incubated with increasing concentrations of AB-^{125}I-LHR$^{96-115}$ (Fig. 2*F*). These results indicate that the photoaffinity labeling is dependent on both the derivatized peptides and hCG as they are limiting factors. In both cases, the derivatized peptides labeled hCGα more than hCGβ, an indication of a labeling specificity.

Labeling Specificity—Specific labeling should be displaced by wild type peptide but not by a peptide that could not bind hCG. We have shown in the previous reports (12), (13) that the Leu^{103} → Ala or Ile^{105} → Ala substitution in LHR abrogated hormone binding. Therefore, Leu^{103} and Ile^{105} were substituted with Ala in LHR$^{96-115}$ to produce a mutant peptide, LHR$^{96-115(L103A/I105A)}$. To test whether the wild type and mutant LHR peptides could inhibit affinity labeling, hCG was incubated with AB-^{125}I-LHR$^{96-115}$ in the presence of increasing concentrations of nonderivatized wild type peptide (Fig. 3*A*) and nonderivatized mutant peptide (Fig. 3*C*). Increasing concentrations of LHR$^{96-115}$ inhibited photoaffinity labeling in a dose-dependent manner and eventually, completely blocked it. These results indicate the specificity of LHR$^{96-115}$ for the photoaffinity labeling. In contrast, the inhibition by mutant LHR$^{96-115(L103A/I105A)}$ was significantly less effective (Fig. 3*C*). Similar results were obtained with affinity cross-linking of ^{125}I-LHR$^{96-115}$ to hCG (Fig. 3, *B*, *D*, and *F*). Although these results indicate the labeling specificity of AB-^{125}I-LHR$^{96-115}$ and ^{125}I-LHR$^{96-115}$, the futile inhibition could be interpreted as the mutant peptide binding to a site in hCG different from the AB-^{125}I-LHR$^{96-115}$ binding site. To test this hypothesis and test whether the mutant peptide could label hCG, LHR$^{96-115(L103A/I105A)}$ was radioiodinated or derivatized and then radioiodinated to prepare ^{125}I-LHR$^{96-115(L103A/I105A)}$ or AB-^{125}I-LHR$^{96-115(L103A/I105A)}$, respectively. As shown in Fig. 4, AB-^{125}I-LHR$^{96-115(L103A/I105A)}$ and ^{125}I-LHR$^{96-115(L103A/I105A)}$ labeled the hCG subunits significantly less. Only trace amounts of labeling were detected, indicating the labeling af-

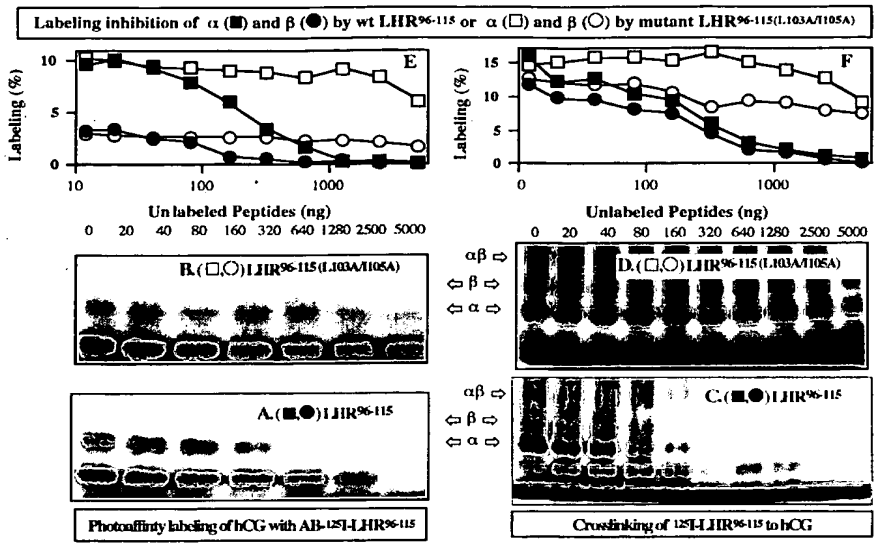

FIG. 3. **Competitive inhibition of affinity labeling by unlabeled wild type peptide and mutant peptide.** AB-^{125}I-LHR$^{96-115}$ was incubated with hCG in the presence of increasing concentrations of wild type LHR$^{96-115}$ (*A*) or mutant LHR$^{96-115(L103A/I105A)}$ (*B*) and irradiated with UV for 30 s. Samples were processed as described in the legend to Fig. 2. In addition, ^{125}I-LHR$^{96-115}$ was incubated with hCG in the presence of increasing concentrations of wild type LHR$^{96-115}$ (*C*) or mutant LHR$^{96-115(L103A/I105A)}$ (*D*) and treated with 0.3 mM SES. The samples were electrophoresed and processed to determine the percent labeling of the hCG α and β subunits. The percent intensities of the labeled α and β bands were determined and plotted against increasing concentrations of unlabeled wild type and mutant peptide (*E* plotted with the percent labeling data from *A* and *C*, and *F* plotted with the percent labeling data from *B* and *D*).

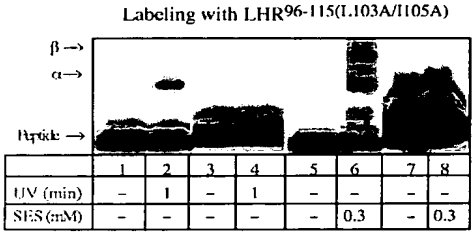

FIG. 4. **Futile affinity labeling of hCG by mutant LHR$^{96-115}$.** hCG was incubated with mutant AB-^{125}I-LHR$^{96-115(L103A/I105A)}$ (*lanes 3* and *4*) or ^{125}I-LHR$^{96-115(L103A/I105A)}$ (*lanes 7* and *8*) and treated with UV for 1 min (*lane 4*) or 0.3 mM SES (*lane 8*), respectively. These samples were processed as described in the legend to Fig. 1. The autoradiograph shows no affinity labeling of hCG as compared with successful labeling of hCG by wild type LHR$^{96-115}$ (*lanes 2* and *6*). *Lanes 1* and *5* show the control hCG samples that were incubated with the wild type peptide but without UV or SES treatment.

finities were significantly low. These results are consistent with the observation that the highest concentrations of nonderivatized LHR$^{96-115(L103A/I105A)}$ slightly attenuated the labeling by AB-^{125}I-LHR$^{96-115}$ and ^{125}I-LHR$^{96-115}$.

Biological Specificity of Affinity Labeling—Although the affinity labeling is specific, our data do not show the biological significance of the affinity labeling. To test this concern, two different experiments were performed. In the first test, denatured hCG was tested for affinity labeling, and in the second the peptides were examined whether they could inhibit ^{125}I-hCG binding to the receptor on intact cells. For the first test, denatured hCG was incubated with increasing concentrations of AB-^{125}I-LHR$^{96-115}$or ^{125}I-LHR$^{96-115}$ and treated with UV or SES, respectively (Fig. 5). Denatured hCG was not labeled at all by either of the LHR peptide derivatives, despite high concentrations of the peptide probes. The results suggest the specificity of the affinity labeling for biologically active hCG. Since SES failed to cross-link ^{125}I-LHR$^{96-115}$ to denatured hCG, ^{125}I-LHR$^{96-115}$ appears to have a difficulty to recognize denatured hCG. To test this possibility, ^{125}I-hCG was incubated with intact cells expressing LHR in the presence of increasing concentrations of the wild type or mutant peptide, LHR$^{96-115}$ or LHR$^{96-115(L103A/I105A)}$ (Fig. 6). The wild type LHR$^{96-115}$ inhibited ^{125}I-hCG binding to the receptor with a K_d value of 43.4 μM, suggesting its binding to the receptor with a reasonable affinity for a peptide (20, 21). In contrast, the K_d value of mutant LHR$^{96-115(L103A/I105A)}$ was 5 mM, which is insignificant. This result, taken together with the futile labeling of denatured hCG (Fig. 5), shows the biological specificity of the binding and labeling of LHR$^{96-115}$ to hCG. Furthermore, the results show that the interaction between hCG and LHR$^{96-115}$ simulates the interaction between hCG and the receptor.

Affinity Labeling Site—LHR$^{96-115}$ has two Lys residues, Lys^{101} and Lys^{112}, which are derivatized with AB or reacted with SES. Since these two Lys are 11 amino acids apart and located in the opposite side of the LRR4 (Fig. 7), it is important to know whether both or only one of them is involved in the affinity labeling. The information will be crucial for defining the orientation and hormone interacting phase of LRR4. To determine the labeling activity of the two Lys residues, one of them was substituted with Ala in LHR$^{96-115}$ to produce LHR$^{96-115(K101A)}$ and LHR$^{96-115(K112A)}$. These two peptides were capable of inhibiting ^{125}I-hCG binding to the receptor on intact cells, but their K_d values were 12–15-fold higher than the corresponding K_d value of the wild type LHR$^{96-115}$ (Fig. 6). The result is consistent with the effect of Ala substitution for Lys^{101} or Lys^{112} in intact receptor on hCG binding. The K_d value for hCG binding of LHR increased by 2.6–3.4-fold when Lys^{101} or Lys^{112} was substituted with Ala (13). Since they were capable of binding hCG, we examined whether the two mutant peptides were also capable of inhibiting photoaffinity labeling of hCG by AB-^{125}I-LHR$^{96-115}$ and affinity cross-linking of ^{125}I-LHR$^{96-115}$ to hCG (Fig. 8). The results show their ability to inhibit the labeling, but the potency was noticeably less than the inhibition potency of the wild type peptide. Again, this result is consistent with the lower affinity of the two mutant peptides to hCG as compared with the affinity of the wild type peptide to hCG. All of these results show their specific interaction with hCG. Finally, we attempted to photoaffinity label hCG with AB-^{125}I-LHR$^{96-115(K101A)}$ and AB-^{125}I-LHR$^{96-115(K112A)}$ (Fig. 9). AB-^{125}I-LHR$^{96-115(K112A)}$ photoaffinity labeled hCG similar to the photoaffinity labeling of hCG by AB-^{125}I-LHR$^{96-115}$, whereas the labeling of hCG with AB-^{125}I-LHR$^{96-115(K101A)}$ was less (Fig. 9*A*). This result indicates that the photoaffinity labeling is significantly more effective when AB is attached to Lys^{101} than to Lys^{112}.

If this is true, one would expect the same trend with affinity cross-linking using the two peptides. Indeed, ^{125}I-

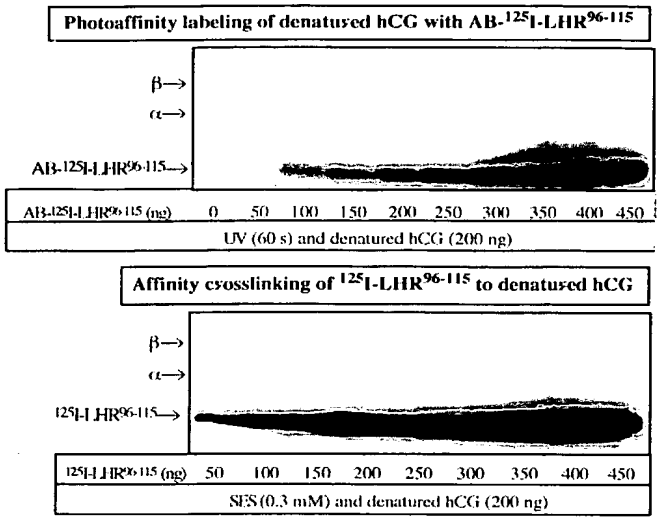

FIG. 5. **Denatured hCG is not affinity-labeled.** Denatured hCG (200 ng) was incubated with increasing concentrations of AB-^{125}I-LHR$^{96-115}$ (*A*) or ^{125}I-LHR$^{96-115}$ and treated with UV for 60 s or 0.3 mM SES (*B*). The samples were processed as described in the legend to Fig. 1.

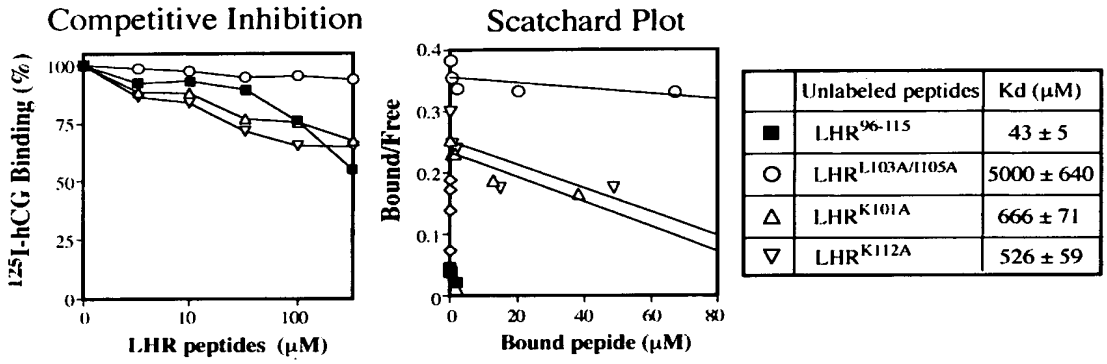

FIG. 6. **Inhibition of ^{125}I-hCG binding to the receptor by LHR peptides.** ^{125}I-hCG was incubated with intact 293 cells expressing LHR in the presence of increasing concentrations of unlabeled wild type and mutant LHR$^{96-115}$ peptides. After washing cells several times to remove unbound ^{125}I-hCG, cells were counted for the bound ^{125}I-hCG as described under "Experimental Procedures." The results were plotted against the concentrations of unlabeled peptides (*left panel*) and converted to Scatchard plots (*right panel*). The K_d values of individual peptides in the table were determined with standard deviations based on the bound/free and bound peptide values as described previously (16).

LHR$^{96-115(K112A)}$ was cross-linked to hCG with SES significantly better than ^{125}I-LHR$^{96-115(K101A)}$ (Fig. 9*B*). However, neither of the derivatized peptides labeled denatured hCG, indicating a specificity of affinity labeling of hCG by AB-^{125}I-LHR$^{96-115(K112A)}$ and ^{125}I-LHR$^{96-115(K112A)}$ (data not shown). Taken together, these results indicate that Lys^{101} is more suitable for affinity labeling hCG than Lys^{112} is. They also suggest that Lys^{101} is at or near the hCG contact point as suggested by the computer model that the short β strand is a ligand contact site, and the Lys^{101} is projected toward ligand (Fig. 7). In contrast, Lys^{112} is located near the α helix as part of the outer lining of the donut structure, at the opposite side from the ligand binding site. Since Lys^{101} is in the N-terminal area of LHR$^{96-115}$, whereas Lys^{112} is in the C-terminal region, one way to verify the conclusion is to use peptide mimics covering the sequences upstream and downstream of LHR$^{96-115}$. To this end, we synthesized two peptide mimics, LHR$^{84-104}$ and LHR$^{113-132}$, and tested them for their ability to inhibit photoaffinity labeling of hCG by AB-^{125}I-LHR$^{96-115}$ and affinity crosslinking of ^{125}I-LHR$^{96-115}$ to hCG (Fig. 10). LHR$^{84-104}$ and LHR$^{113-132}$ inhibited the affinity labeling of hCG, but their potency was less than that of LHR$^{96-115}$. LHR$^{84-104}$ was more effective in inhibiting hCGα than LHR$^{113-132}$ was. On the other hand, LHR85–104 was similar to LHR$^{113-132}$ in inhibiting the labeling of hCGβ.

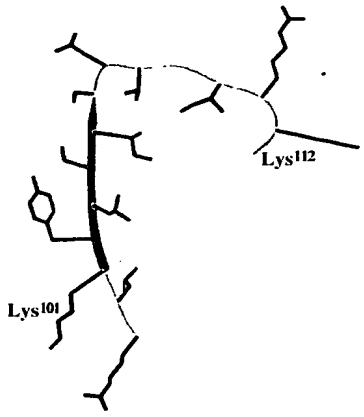

FIG. 7. **Model of LRR4.** Lys^{101} and Lys^{112} in LRR4 are projected on the opposite side of LRR4 (13).

Interaction of LHR$^{96-115}$-hCG Complex with Exoloops—In the preceding article (13), we pointed out the absolute homology in the 8 residues (boldface) in the **Leu**^{98}-**Pro**-Gly-**Leu**-Lys-Tyr-**Leu**-Ser-**Ile**-Cys-**Asn**-**Thr**-**Gly**^{109} sequence among cloned LHR, follicle-stimulating hormone receptor, and thyroid-stim-

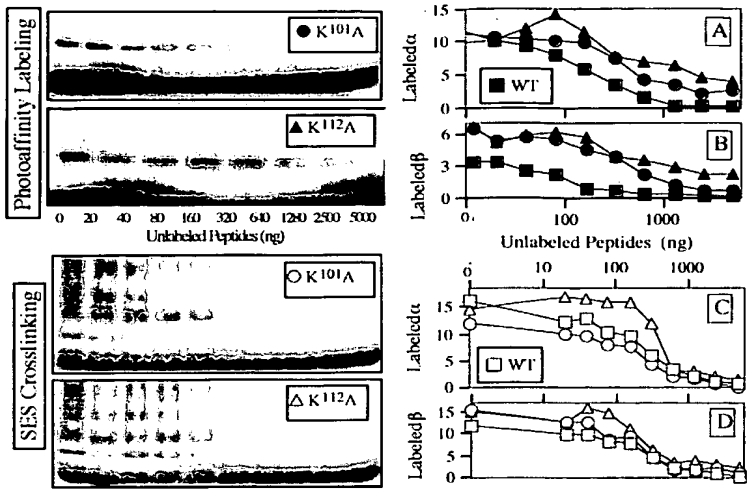

FIG. 8. **Inhibition of affinity labeling by Lys to Ala mutant peptides.** hCG was affinity-labeled with AB-^{125}I-LHR$^{96-115}$ and ^{125}I-LHR$^{96-115}$ in the presence of increasing concentrations of LHR$^{96-115(K101A)}$ or LHR$^{96-115(K112A)}$ as described in the legend to Fig. 3. After processing the samples, the phosphoimage of the gel was analyzed to determine the percent labeling the hCG subunits. The results were plotted against the concentration of peptides for the inhibition of photoaffinity labeling hCGα (*A*) and hCGβ (*B*) and affinity cross-linking to hCGα (*C*) and hCGβ (*D*). In addition, the inhibition of unlabeled wild type LHR$^{96-115}$ was presented for comparison.

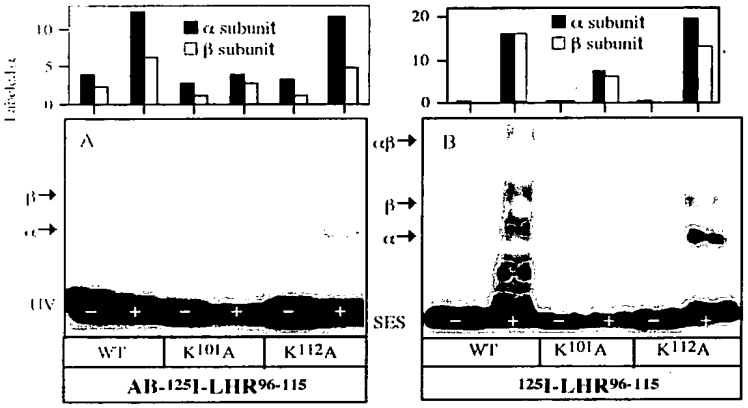

FIG. 9. **Affinity labeling of hCG with Lys to Ala mutant peptides.** *A*, hCG was photoaffinity-labeled with AB-^{125}I-LHR$^{96-115}$, AB-^{125}I-LHR$^{96-115(K101A)}$, or AB-^{125}I-LHR$^{96-115(K101A)}$ as described in the legend to Fig. 3. *B*, likewise, hCG was affinity-cross-linked to ^{125}I-LHR$^{96-115}$, ^{125}I-LHR$^{96-115(K101A)}$, or ^{215}I-LHR$^{96-115(K101A)}$ as described in the legend to Fig. 3. "+" and "−" indicate the treatment with or without UV or SES.

ulating hormone receptor of various species. Furthermore, we showed that the tandem three conserved residues, **Asn**^{107}-**Thr**-**Gly**^{109}, were more important for cAMP induction than hormone binding. This is unique because the exodomain is responsible of high affinity hormone binding and mutations in the exodomain impact hormone binding, which in turn affected cAMP induction, not the other way around. Therefore, we have raised the possibility that this region may be involved in the interaction with the endodomain and, thus, in signal generation. This is a crucial issue, because the exodomain and endodomain are known to interact (22–25), and this interaction regulates the generation of hormone signals (22), (23). However, the exact contact points in the exodomain and endodomain are unknown. Since the three exoloops in the endodomain are a logical candidate for the exodomain/endodomain interaction, we have synthesized peptide mimics for the exoloops 1, 2, and 3 of LHR (LHR^{exo1}, LHR^{exo2}, and LHR^{exo3}) and tested whether they could inhibit the photoaffinity labeling of hCG by AB-^{125}I-LHR$^{96-115}$ (Fig. 11). LHR^{exo2} effectively inhibited the photoaffinity labeling, whereas the inhibition by LHR^{exo1} was less. In contrast, LHR^{exo3} did not inhibit the labeling. These differential effects suggest the specificity of the inhibition.

DISCUSSION

Our results show that AB-^{125}I-LHR$^{96-115}$ photoaffinity labels hCG. Ample evidence is presented to support the specificity of the photoaffinity labeling under rigorous conditions. The labeling is saturable and dependent on the hCG concentration, derivatized ^{125}I-LHR$^{96-115}$ concentration, and UV activation. AB-^{125}I-LHR$^{96-115}$ photoaffinity labels bioactive hCG but not denatured hCG. This labeling is blocked by nonderivatized wild type LHR$^{96-115}$ but not by nonderivatized mutant LHR$^{96-115(L103A/I105A)}$. The same Ala mutations in LHR abolish the hCG binding activity of LHR. Furthermore, AB-^{125}I-LHR$^{96-115(L103A/I105A)}$ does not photoaffinity label bioactive hCG and denatured hCG. LHR$^{96-115}$ inhibits ^{125}I-hCG binding to the receptor expressed on intact cells but LHR$^{96-115(L103A/I105A)}$ is not capable of inhibiting ^{125}I-hCG binding to the receptor. To avoid the potential interference of the photoactivable group on binding of AB-^{125}I-LHR$^{96-115}$ to the receptor and the subsequent labeling, ^{125}I-LHR$^{96-115}$ was affinity-cross-linked to hCG with SES. This affinity labeling is equally successful with similar specificity.

Both subunits of hCG are labeled, indicating that the UV-activable group coupled to AB-^{125}I-LHR$^{96-115}$ can reach them. This is consistent with other studies (26–28) and not surprising, since the two subunits are closely intertwined in the crystal structure (29, 30). Interestingly, hCGα was preferentially labeled. Obviously, the reagent more readily reaches and labels the α subunit than the β subunit. Since the maximum labeling distances of AB is 7 Å (19), hCGα is likely to contact AB-^{125}I-LHR$^{96-115}$. Our results are inconsistent with the unlikely possibility that the peptide associates with hCG at sites other than the receptor contact site, impacts the global structure of hCG, and interferes with the hormone/receptor interaction. Since

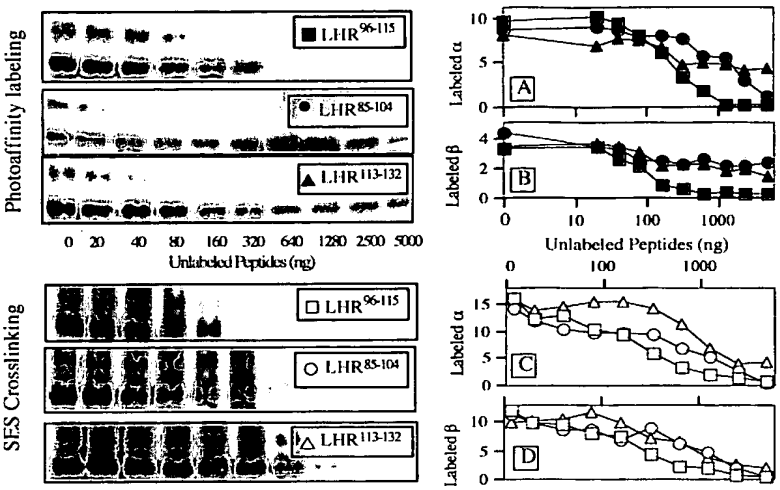

FIG. 10. **Roles of peptides flanking LHR^{96-115} on affinity labeling.** hCG was photoaffinity-labeled with AB-^{125}I-LHR^{96-115} and affinity-cross-linked to ^{125}I-LHR^{96-115} with SES in the presence of increasing concentrations of LHR^{96-115}, LHR^{85-104}, or $LHR^{113-132}$ as described in the legend to Fig. 3. After processing the samples, the percent labeling of hCGα and hCGβ was determined. The results are presented for the inhibition of photoaffinity labeling of hCGα (*A*) and hCGβ (*B*) and the inhibition of affinity cross-linking of hCGα (*C*) and hCGβ (*D*).

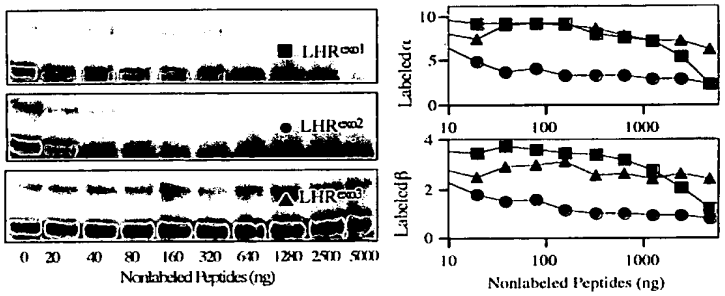

FIG. 11. **Roles of exoloop peptides on affinity labeling.** hCG was photoaffinity-labeled with AB-^{125}I-LHR^{96-115} in the presence of increasing concentrations of LHR peptide mimics corresponding to the sequences of exoloops 1, 2, and 3 (LHR^{exo1}, LHR^{exo2}, and LHR^{exo3}) as described in the legend to Fig. 3. Exoloop 1 connects transmembrane domains 2 and 3, exoloop 2 connects TMs 4 and 5, and exoloop 3 connects transmembrane domains 6 and 7. The LHR^{exo1} sequence is Asp-Ser-Gln-Thr-Lys-Gly-Gln-Tyr-Tyr-Asn-His-Ala-Ile-Asp-Trp-Gln-Thr-Gly-Ser-Gly-Cys-Ser-Thr. The LHR^{exo2} sequence is Ser-Asn-Tyr-Met-Lys-Val-Ser-Ile-Cys-Phe-Pro-Met-Asp-Val-Glu-Thr-Thr-Leu-Ser-Gln. LHRexo3 comprises Lys-Val-Pro-Leu-Ile-Thr-Val-Thr-Asn-Ser-Lys. Labeled hCG was processed, and the percent labeling of hCGα and hCGβ was determined as described in the legend to Fig. 3.

LHR^{96-115} inhibits hCG binding to the receptor, AB-^{125}I-LHR^{96-115} interacts with hCG at or near a contact site of hCG and the LH/CG receptor.

It is significant that only one of the hCG$\alpha\beta$ subunits, but not both, is labeled, although two AB could be attached to the two Lys residues of LHR^{96-115}. This suggests that only one of the Lys residues is close to hCG. Indeed, photoaffinity labeling using mutant peptides lacking one of the Lys residues shows that the AB coupled to Lys^{101} is capable of labeling hCG, whereas the AB attached to Lys^{112} is less effective. This is strong evidence to support the orientation of Lys^{101} and Lys^{112} in the LRR4 loop model (Fig. 7) and implicates the N-terminal region of LHR^{96-113}, including the putative β strand of LRR4, in the interaction with hCG.

The crystallization of Leu-rich repeats (11, 31) and their presence in the middle of the exodomain of all glycoprotein hormone receptors (1) generated much speculation (8, 32–34) that the eight to nine LRRs provide the primary contact site for the cognate ligands, LH/CG, FSH, and TSH. They comprise the bulk of the exodomain at its center and are computer-modeled to show a crescent structure. The inner surface of the crescent consists of β sheets of the repeats and is thought to be the ligand contact site (8, 31, 32), perhaps interacting with the putative receptor binding αC terminus and seat belt side of hCG (29). However, little experimental evidence has been available to support these popular views. Our results of this and the preceding articles (12, 13) are the first experimental evidence supporting the LRR structure of LHR and the direct interaction of the LRR4 β strand with hCG. Our studies have laid the ground work to determine the contact residues of the receptor and the hormone.

It has been known that LHR interacts hCG initially at the exodomain, and the exodomain-hCG complex impacts the endodomain. This secondary contact is thought to generate the hormone signals (22, 23). There is evidence that the exodomain and endodomain are intimately associated before and after hormone binding (24, 25). This association is crucial because it affects the hormone binding affinity and provides a mechanism for the signal generation (24, 25). Unfortunately, there are few clues to the site of the interaction between the exodomain and endodomain except the recent reports implicating exoloops 2 and 3 (24, 25). The observations described in this and preceding articles (12, 13) show the involvement of LRR4 in the signal generation, implicating exoloop 2 and, perhaps, exoloop 1 as contact points of the exodomain/hCG complex. In fact, our computer modeling shows that the exoloop 2 projects straight up from the connecting the transmembranes 4 and 5, like a hairpin, toward the exodomain. It will be interesting to see whether the hairpin structure of exoloop 2 interacts with the crescent LRR structure of the exodomain, in particular LRR4. Such an exodomain/endodomain interaction could provide a

mechanism for the mutual modulation of the two distinct domains (24, 25) and signal generation.

REFERENCES

1. McFarland, K., Sprengel, R., Phillips, H., Kohler, M., Rosemblit, N., Nikolics, K., Segaloff, D., and Seeburg, P. (1989) *Science* **245,** 494–499
2. Loosfelt, H., Misrahi, M., Atger, M., Salesse, R., Thi, M., Jolivet, A., Guiochon-Mantel, A., Sar, S., Jallal, B., Garnier, J., and Milgrom, E. (1989) *Science* **245,** 525–528
3. Tsai-Morris, C. H., Buczko, E., Wang, W., and Dufau, M. L. (1990) *J. Biol. Chem.* **265,** 19385–19388
4. Xie, Y. B., Wang, H., and Segaloff, D. L. (1990) *J. Biol. Chem.* **265,** 21411–21414
5. Ji, I., and Ji, T. H. (1991) *Endocrinology* **128,** 2648–2650
6. Koo, Y. B., Ji, I., Slaughter, R. G., and Ji, T. H. (1991) *Endocrinology* **128,** 2297–2308
7. Moyle, W., Campbell, R., Rao, S., Ayad, N., Bernard, M., Han, Y., and Wang, Y. (1995) *J. Biol. Chem.* **270,** 20020–20031
8. Jiang, X., Dreno, M., Buckler, D., Cheng, S., Ythier, A., Wu, H., Hendrickson, W., and Tayar, N. (1995) *Structure (Lond.)* **3,** 1341–1353
9. Couture, L., Naharisoa, H., Grebert, D., Remy, J. J., Pajot-Augy, E., Bozon, V., Haertle, T., and Salesse, R. (1996) *J. Mol. Endocrinol.* **16,** 15–25
10. Bhowmick, N., Huang, J., Puett, D., Isaacs, N. W., and Lapthorn, A. J. (1996) *Mol. Endocrinol.* **10,** 1147–1159
11. Kobe, B., and Deisenhofer, J. (1994) *Trends Biochem. Sci.* **19,** 415–421
12. Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) *J. Biol. Chem.* **276,** 3426-3435
13. Song, Y., Ji, I., Beauchamp, J., Isaacs, N., and Ji, T. (2001) *J. Biol. Chem.* **276,** 3436-3442
14. Ji, I., and Ji, T. H. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 7167–7170
15. Ryu, K.-S., Gilchrist, R. L., Ji, I., Kim, S.-J., and Ji, T. H. (1996) *J. Biol. Chem.* **271,** 7301–7304
16. Levitzki, A. (1984) *Receptors: Quantitative Approach*, pp. 19–26, Benjamin/Cummings Publishing Co., Menlo Park, CA
17. Ji, T. H. *(1985) Methods Enzymol.* **91,** 580–609
18. Ji, T. (1979) *Biochim. Biophys. Acta* **559,** 39–69
19. Ji, I., and Ji, T. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 5465–5469
20. Phang, T., Kundu, G., Hong, S., Ji, I., and Ji, T. H. (1998) *J. Biol. Chem.* **273,** 13841–13847
21. Roche, P. C., Ryan, R. J., and McCormick, D. J. (1992) *Endocrinology* **131,** 268–274
22. Ji, T. H., Murdoch, W. J., and Ji, I. (1995) *Endocrine* **3,** 187–194
23. Ji, T. H., Grossmann, M., and Ji, I. (1998) *J. Biol. Chem.* **273,** 17299–17302
24. Ryu, K., Lee, H., Kim, S., Beauchamp, J., Tung, C., Isaacs, N. W., Ji, I., and Ji, T. H. (1998) *J. Biol. Chem.* **273,** 6285–6291
25. Ryu, K., Gilchrist, R. L., Tung, C., Ji, I., and Ji, T. H. (1998) *J. Biol. Chem.* **273,** 28953–28958
26. Morbeck, D. E., Roche, P. C., Keutmann, H. T., and McCormick, D. J. (1993) *Mol. Cell. Endocrinol.* **97,** 173–181
27. Liu, C., Roth, K. E., Shepard, B. A., Shaffer, J. B., and Dias, J. A. (1993) *J. Biol. Chem.* **268,** 21613–21617
28. Cosowsky, L., Lin, W., Han, Y., Bernard, M., Campbell, R., and Moyle, W. (1997) *J. Biol. Chem.* **272,** 3309–3314
29. Lapthorn, J. P., Harris, D. C., Littlejohn, A., Lustbader, J. W., Canfield, R. E., Machin, K. J., Morgan, F. J., and Isaacs, N. W. (1994) *Nature* **369,** 455–461
30. Wu, H., Lustbader, J. W., Liu, Y., Canfield, R. E., and Hendrickson, W. A. (1994) *Structure (Lond.)* **2,** 545–558
31. Kobe, B., and Deisenhofer, J. (1995) *Nature* **374,** 183–186
32. Remy, J. J., Couture, L., Pantel, J., Haertle, T., Rabesona, H., Bozon, V., Pajot-Augy, E., Robert, P., Troalen, F., Salesse, R., and Bidart, J. M. (1996) *Mol. Cell. Endocrinol.* **125,** 79–91
33. Puett, D., Bhowmick, N., Fernandez, L. M., Huang, J., Wu, C., and Narayan, P. (1996) *Mol. Cell. Endocrinol.* **125,** 55–64
34. Thomas, D., Rozell, T., Liu, X., and Segaloff, D. (1996) *Mol. Endocrinol.* **10,** 760–768